

USSN: 10/521,693
Attorney Docket: I-2002.011 US
Response to Office Action of January 19, 2007

Amendments to the Specification:

Please replace the paragraph on page 5, lines 26-28 with the following paragraph:

The level of nucleotide homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTN" that can be found at the U.S. Department of Health and Human Services' National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information internet site www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html.

Please replace the paragraph on page 12, lines 11-17 with the following paragraph:

The level of protein homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTP", that can be found at the U.S. Department of Health and Human Services' National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information internet site www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html. A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Matrix used: "blosum62". Parameters used are the default parameters:

Open gap: 11. Extension gap: 1. Gap x_dropoff: 50.

Please replace the paragraph on page 17, lines 8-23 with the following paragraph:

Methods for large-scale production of antibodies according to the invention are also known in the art. Such methods rely on the cloning of (fragments of) the genetic information encoding the protein according to the invention in a filamentous phage for phage display. Such techniques are described i.a. at the "Antibody Engineering Page" under "filamentous phage display" at

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<http://axim1.imt.uni-marburg.de/~rek/aepphage.html>, and in review papers by Cortese, R. et al., (1994) in Trends Biotechn. 12: 262-267., by Clackson, T. & Wells, J.A. (1994) in Trends Biotechn. 12: 173-183, by Marks, J.D. et al., (1992) in J. Biol. Chem. 267: 16007-16010, by Winter, G. et al., (1994) in Annu. Rev. Immunol. 12: 433-455, and by Little, M. et al., (1994) Biotechn. Adv. 12: 539-555. The phages are subsequently used to screen camelid expression libraries expressing camelid heavy chain antibodies. (Muyldermans, S. and Lauwereys, M., Journ. Molec. Recogn. 12: 131-140 (1999) and Ghahroudi, M.A. et al., FEBS Letters 414: 512-526 (1997)). Cells from the library that express the desired antibodies can be replicated and subsequently be used for large scale expression of antibodies.

Please replace the paragraph on page 21, lines 12-15 with the following paragraph:

A special form of such a vehicle, in which the antigen is partially embedded in the vehicle, is the so-called ISCOM (EP 109.942, EP 180.564, EP 242.380)

In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. Span or Tween: SPAN or TWEEN compositions.

Please replace the paragraph beginning on page 28, line 15 extending to page 29, line 3 with the following paragraph:

Chromosomal DNA from *B. hyodysenteriae* strain B204 was isolated by standard methods and digested with HindIII according to the manufacturers directions. The restriction enzyme was removed by phenol extraction and ethanol precipitation. 5 μ g of the digested chromosomal DNA was mixed with 0.1 μ g of HindIII digested pBluescript pBLUESCRIPT® II SK vector (Stratagene Co.). To this mixture 5 units of T4 DNA ligase (Gibco-BRL) was added and incubated for 18

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hours at 14°C. *E. coli* strain DH5 α was made competent (Hanahan) and transformed with 10 μ l of the ligation mix and after recovery for 1 hour at 37°C in Luria Bertani broth the mixture was plated on Luria Bertani plates supplemented with 100 μ g/ml of ampicillin. A total of >4000 colonies was obtained. A total of 4000 colonies from the obtained HindIII library were plated on LB plates with 100 μ g/ml of ampicillin and grown for 18 hours at 37°C. Colonies were lifted onto nitrocellulose filters, fixed in chloroform vapor and then lysed with SDS using standard methods (Sambrook *et al.*). The obtained filters were incubated with serum obtained from a pig with *B. hyodysenteriae* infection. The colony blots were developed with rabbit-anti pig alkaline phosphatase conjugated secondary antibodies. Colonies reacting with the convalescent serum were purified and plasmid DNA was extracted from cultures grown in LB medium with 100 μ g/ml of ampicillin.

Please replace the paragraph beginning on page 30, line 7 extending to page 31, line 3 with the following paragraph:

PCR was used to amplify the genes encoding the mature length proteins BlpB and BlpC using primers designed to engineer unique restriction endonuclease sites into the final product. The 5' primers incorporate either an *Nde*I site or a *Bam*H1 site. The 3' primers incorporate a *Bam*HII site or an *Nde*I site. An aliquot of the PCR products were visualized on an agarose gel and the remaining portions were digested with *Bam*HI (Roche) and/or *Nde*I (Roche) according to manufactures instructions. Restriction endonuclease were inactivated by phenol extraction and the digested PCR products were purified by ethanol precipitation. The pET-15b vector was digested and purified as per the PCR products and treated with alkaline phosphatase. 150 μ g of the digested PCR products were ligated into 80 ng of digested pET15-b using T4 DNA ligase (Roche) according to manufactures instructions. The ligations were transformed into *E. coli*

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DH5 α . Clones containing single inserts were identified using colony PCR with primers designed to flank the multicloning site of the vector: 5'-TAA-TAC-GAC-TCA-CTA-TAG-G-3' and 5'-GGA-AAC-AGC-TAT-GAC-CAT-G-3'. For each ligation the insert of one of the clones was checked by double stranded DNA sequencing. Plasmid DNA was isolated from the positive clone and transformed into *E. coli* strain BL21(DE3)pLysS for expression. To check expression of recombinant proteins whole cell lysates of the induced cultures of the expression clones were compared with the expression strain harboring a pET15b plasmid without an insert. Whole cell lysates were separated by SDS-PAGE (as described previously) and stained with coomasic brilliant blue (CBB). Bands corresponding to the predicted molecular weights of all the recombinant proteins were observed except for BlpB. By transformation of the blpB.pET15b construct into CodonPlus CODONPLUS® RIL (Stratagene) strain a band corresponding to its molecular weight was also observed.

Please replace the paragraph beginning on page 31, lines 6-23 with the following paragraph:

To check whether the recombinant proteins were recognized by convalescent sera whole cell lysates of the induced cultures of the clones expressing BlpB and BlpC were separated by SDS-PAGE alongside the expression strain harboring a pET15b plasmid without an insert. Proteins were then transferred onto Immobilon-P IMMOBILON-P membranes (Millipore) with a Trans-Blot® TRANS-BLOT® Electrophoretic transfer Cell (Bio-Rad) for 1 hour at 100 V according to the manufacturers instructions. After transfer, the membranes were blocked by soaking in 5% skim milk buffer [5% (w/v) skim milk powder in TBS-Tween-20 TBS-TWEEN 20 (0.15 M NaCl, 0.05 M Tris-HCl pH 7.4, 0.05% Tween-20 TWEEN 20)] for 1 hr. Serum obtained from a pig with *B. hyodysenteriae* infection was diluted 1/100 in skim milk buffer, and incubated overnight. Three 5-minute washes in TBS-Tween TBS-TWEEN were performed before incubating the membranes with the secondary antibody [1/400 dilution of horse-radish peroxidase (HRPO) – conjugated rabbit-anti pig IgG (Sigma)]. The secondary antibody was

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diluted in skim milk buffer and incubated with the membranes for 2 hours at 37°C with shaking, followed by three 5-minute washes as above, with the addition of a final 5 minute wash in TBS. The membranes were developed using HRPO substrate [4-chloro-1-naphthol (Merck), 100% methanol, 30% (w/v) H₂O₂ TBS].

Please replace the paragraph beginning on page 31, line 28 extending to page 32, line 13 with the following paragraph:

Cultures of strains expressing recombinant BlpC were grown to an optical density of 0.6 at 600nm and induced for 4 hrs with 10mM isopropylthio-β-D-galactoside (IPTG; Sigma). Cultures were lysed using a French pressure cell and recombinant proteins were purified using Talen TALON resin (Clontech) by immobilised metal affinity chromatography (IMAC) according to the manufacturers instructions. The column eluates were pooled and dialysed overnight against phosphate buffered saline and concentrated to a 0.5 mL volume using Centicon-10 (Millipore) concentrators. The concentration of sample was determined by Bradford assay. Freund's incomplete adjuvant was combined in equal volumes with 100 µg of each of the recombinant proteins. Two New Zealand White rabbits were injected subcutaneously with 50 µg of purified recombinant protein. After 4 weeks the rabbits were injected with another 50 µg of purified recombinant protein intradermally. After an additional week the rabbits were subjected to a terminal bleed by cardiac puncture. The rabbit antisera were shown to react with recombinant protein in Western blotting experiments at dilutions at 1/2000.